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Phenotypic Characteristics of Colletotrichum Species Associated with Mango (Mangifera Indica L.) in Southwest Ethiopia

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Abstract
Anthracnose of mango caused by Colletotrichum species is among the numerous diseases causing low production and quality of mango fruit in Ethiopia. The objectives of this work were to identify and characterize Colletotrichum species isolates responsible for anthracnose of mango in Southwest of Ethiopia. Samples of infected mango leaves, panicles and immature fruits were collected from home gardens of nine districts in Southwestern part of Ethiopia. Among them eight isolates of Colletotrichum species with distinct morphology on PDA were observed in each group. Colony color, shape and diameter of every culture were recorded, conidial size and shapes were computed from 20 conidia per isolate. The results showed that f Colletotrichum species isolates were grouped into three distinct morphological types: Colletotrichum gloeosporioide morph type I (37%) with hyaline cylindrical conidia rounded both ends, Colletotrichum acutatum morphotype II (38%) conidia mass in the center and fusiform tapered to a point in both ends, and Colletotrichum asianum morph type III (25%) cylindrical conidia with obtuse to slightly rounded ends. The length of conidia ranged from 10.5-17.8 µm, width (3.22 to 6.9 µm). Among six media tested, highest mean colony diameter of 51.9 mm was recorded on Potato dextrose agar and the lowest mean mycelial growth of 18.4 mm was recorded on Tap water agar. All isolates had good sporulating capacity on general media. Based on the results of this study, it could be concluded that C. gloeosporioides, C. acutatum and C asianum were found to be the major causal agents of mango anthracnose. Additional study on the epidemiology of anthracnose of mango is needed for further disease management strategies.
Keywords: Anthracnose, *Colletotrichum* spp., identification, mango, epidemiology

INTRODUCTION

Mango (*Mangifera indica* L) is an important fruit crop in most tropical regions of the world and most consumed in the developed countries (Diedhiou *et al*., 2007). The dietary contributions of mango fruit in the tropics rank above that of citrus fruits. Mangoes account for approximately half of all tropical fruits produced worldwide (Phoulivong *et al.,* 2010).

The worldwide production of mango was estimated at nearly 39 million tons in 2009 (FAO, 2010). The amount of mango production in Africa this year was 13.6 million tons (FAO, 2010) In Ethiopia mango is produced mainly in western, Southwestern and eastern Regional States of the country : Oromia, Southern Nations Nationalities, and People Region, Benishangul Gumz and Amhara Regional State (Yeshitela and Nessel, 2004; Desta, 2005; Chala *et al*., 2014). The average production of mango in Ethiopia is estimated to be 11,446.2 ton per annual (FAO, 2010), and its more area coverage is in the South-western Ethiopia. Mango production and supply in Ethiopia is fluctuating, because of occurrence of diseases (Ayantu *et al*., 2014).

Mango is affected by a number of diseases at all stages of development, from seedling in the nursery to the fruit in storage or transit (Ploetz, 2003; Prakash, 2004). Anthracnose is a major pre - and post - harvest disease of mango fruit, causing direct yield loss in the field, pack house, and market. The genus Colletotrichum is recently designated as the world's eighth most important group of plant pathogenic fungi based on perceived scientific and economic importance (Hyde *et al*., 2009b; Phoulivong *et al*., 2010; Dean *et al.,* 2012) which cause fruit damage and production losses.

Colletotrichum affects the leaves, flowers, panicles, and fruits of mango trees causing anthracnose worldwide. In areas where rain is prevalent during flowering and fruit set, anthracnose can cause destruction of the inflorescences and infection and drop of young fruits where this can obviously lead to serious losses, reaching up to 35% of the harvested fruits (Martinez *et al.,* 2009).

Mango anthracnose is caused by *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*), but in some cases *C. acutatum* (teleomorph: *Glomerella acutatum)* has also been reported as a cause of the disease (Peres *et al.* 2005; Jayasinghe and Fernando, 2009; Phoulivong *et al.,* 2010). Identification and characterization of Colletotrichum species is based on morphological characters such as size and shape of conidia and appressoria, existence of setae or presence of a teleomorph, and cultural characters such as colony color, growth rate and texture (Smith and Black, 1990).

Even though mango fruit is economically and nutritional important in Ethiopia, information on anthracnose disease of mango is scant. Pathogens infecting mango fruit in Ethiopia including the current study area have not yet been characterized, and not fully documented although diseases affecting the crop in the

country have been reported based mainly on field symptoms.

Thus, the importance of anthracnose on mango in Ethiopia dictates the need for investigation on the identification and characterization of Colletotrichum isolates to provide useful information about Colletotrichum isolates and suggest strategies to prevent and control pathogen. Thus, the aims of this study were to identify and characterize Colletotrichum species associated with anthracnose symptoms on mango tissues found in the different locations of Southwest Ethiopia.

MATERIALS AND METHODS

Descriptions of the study area

A study was carried out in Southwestern part of Ethiopia in different agro-ecological zones, during 2013-2014 cropping season. The study area is located at 7^0 12'N--9^0 6'N Latitude and 35^0 27'E--37^0 09'E longitude with altitude ranging from 1000-2500m a.s.l. Samples of infected mango leaves, panicles and fruits were collected from mango home gardens in nine districts and brought to the Jimma University College of Agriculture and Veterinary Medicine, plant pathology laboratory. At the laboratory, portions of peeled epicarp and flesh of the infected panicle, immature fruit and leaves were removed at the point of progression of disease symptom; cut into small pieces and then soaked in 70 per cent ethanol solution for 3 minutes, 2 percent sodium hypochlorite (NaOCl) for another 3 minutes, then rinsed in two changes of sterile distill water. The parts were, dropped on sterile paper towels, allowed dried before plating them onto Potato Dextrose Agar (PDA) and incubate for 5 days at room temperature; isolated colonies were sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examinations and viewing under stereo and compound light microscopes. They were described and classified based on conidia and colony morphology as described by Dugan *et al*. (2006).

Identification of the pathogen

The pathogens were identified based on their cultural and morphological characters. A loop full of fungal culture grown on PDA plates were taken on a glass slide and observed with image analyzer under 100 X magnifications for the presence of conidia and conidiophores. After confirming the spores, the cultures were purified by single spore isolation technique. The funguses were identified on the basis of morphological characteristics as suggested by Agron, (2009) and Ellis (2009).

Following Sivakumar *et al*. (1997) method, suspension of conidia were prepared by suspending mycelia scraped from seven days old of the eight most frequently recovered Colletotrichum spp isolate by separately putting in 3-milliliter sterile distilled water and shaking vigorously for 3 minutes. The resulting suspensions were filtered through 2-layer cheesecloth. The concentrations of spore suspension were adjusted to 10^6 spores or conidia by using haemacytometer.

Maintenance of the culture: the fungus were sub- cultured on PDA slant and allowed to grow at 27 ± 1° C for 12 days. Such slants were preserved in refrigerator at 4° C and renewed once in a month. These pure cultures were used for characterization.

Phenotypic Characteristic of Colletotrichum species Isolates.

Three mm plugs were aseptically punched from actively growing edge of a 5-day-old culture of these isolates. Each plug was placed onto PDA Petri dishes and incubated after 7 days, colony size, shape, margin and color were recorded. Colony color (mycelia) observed upper side and types of pigments from the reverse side of each Colletotrichum spp isolates were determined on different media using RGB color chart (Anonymous, 2005). Colony diameter of every culture was recorded daily for 7 days. Growth rate was calculated as the 7-day average of mean daily growth (mm per day).

Three cultures plate of each isolate were investigated and experiments were conducted twice.

For examination of conidial morphology, all isolates were sub-cultured as mentioned above. Cultures were washed with sterile water and drops of the suspension were placed on microscope slides and mixed with lacto phenol/cotton blue to stain the conidia. Conidial size (length and width) were computed from 20 conidia per isolate. Length and width of conidia were measured with ocular micrometer (µm), which were fitted into 10x eyepiece. Colletotrichum spp. were classified in three classes, according to their morphology of conidia: 0 (conidia rounded on both ends); 1 (1 round, 1 sharp ended conidia) and 2 (both end sharpened conidia) (Sutton, 1992).

Sporulation capacity ten days old cultures of each Colletotrichum spp. isolates, incubated on different media were washed by flooding with 10 ml sterilized distilled water, rubbed with sterilized scalpel and transferred to 50 ml sterilized beaker and thoroughly stirred for 10-15 minutes with magnetic stirrer to extract the spores from the interwoven mycelia and then filtered into another sterilized beaker through double layer cheese clothes.

Cultural Characters of the Isolates of Colletotrichum spp. on Different Solid Media

Different solid media mentioned below were used for assessing the growth of isolates of Colletotrichum spp. The mycelia diameters as well as morphological character of mycelia on different media were recorded.

Mango leaves extract agar (MLEA) Host leaves 200g, agar-agar 20 g and distilled water 1000 ml two hundred grams of mango leaves were cut into small bits, boiled in 500 ml distilled water for 30 minutes and extract were collected by filtering through cheesecloth. Agar-agar 20 g was dissolved in the leaves extract and the final volume were made up to 1000 ml with distilled water and sterilized. Mango fruit extract agar (MFEA): Host fruit 200g, agar-agar 20g and distilled water 1000 ml, two hundred grams of mango fruit were cut into small bits, boiled in 500 ml distilled water for 30 minutes and extract were collected by filtering through cheesecloth. Agar-Agar 20 g were dissolved in the leaf extract and the final volume were made up to 1000 ml with distilled water and sterilized. Sabouraud dextrose agar, Dextrose ($C_6H_{12}O_6$) 40 g, Peptone 10 g, Agar-agar 20 g, Distilled water 1000 ml all the ingredients were dissolved one by one in 400 ml distilled water and agar were dissolved separately in 500 ml distilled water and mixed with the above solution and the volume were made up to one litter and sterilized as described earlier. Tap water agar (TWA) 1000ml with Agar-agar 20 g, and sterilized. Malt extract agar (MEA): Malt extract: 25 g Agar- agar: 20 g Distilled water (to make up): 1000 ml Malt extract was dissolved in 400 ml of distilled water. Agar- agar was melted separately in 400 ml of distilled water. Both solutions were mixed thoroughly and final volume was made up to 1000 ml with distilled water and autoclaved.

Pathogenicity test

The isolates used in morphological characterization were selected for pathogenicity tests on detached fruits, and leaves of mango, Preparation of hosts freshly harvested untreated, physiologically mature and unripe fruits was collected from the mango field of Malkassa Agricultural Research Center variety Kent. The detached fruits and leave were washed under running tap water for 60 seconds followed by surface sterilization by immersing the fruits in 70% ethanol for 3 minutes, 2% sodium hypochlorite solution for 5 minutes and then rinsing three times in sterile distilled water for 2 minutes and drying with sterile tissue paper and then air drying (Sanders and Korsten, 2003). Based on the morphological characterization, eight isolates were selected for inoculation on detached mango fruit, and leaves. Surface sterilized fruits and leaves were placed in a plastic box with tissue paper then sprayed with sterilized water to maintain at least 95% relative humidity (Than *et al*. 2008a). The samples were inoculated using the wound/drop inoculation method (Lin *et al*. 2002) which included pin-pricking on leaves, the fruits to a 3 mm depth with a sterile needle in the middle portion of fruit and then placing 20µl of conidia suspension onto the wound (Freeman and Shabi 1996, Than *et al.* 2008a, b). Control fruits were inoculated with 20µl of sterile distilled water. The inoculated samples were incubated in the plastic containers at 25°C under controlled conditions. The plastic box removed after 48hr and fruits and leaves were kept at the same temperature. The causative organisms in the diseased parts were re-isolated on potato dextrose agar as described in isolation of pathogen. The characters of the re-isolated pathogens were compared with their original isolates.

Design and Data analysis

The laboratory experimental units were three replicates and the Completely Randomized Design (CRD) was used. The experiment was repeated twice. The generated data were analyzed using SAS version 9.2 software. Mean values among treatments were compared by the LSD at $\alpha = 0.05\%$ level of significance.

RESULTS

Phenotypic Characteristics of Colletotrichum Species Isolate

The identified Colletotrichum species isolates were grouped into three morphological types *Colletotrichum gloeosporioide* (morphotype I. isolate 1 to 3), *Colletotrichum acutatum* (morphotype II. isolate 4 to 6) and *Colletotrichum asianum* (morph type III, isolate 7 and 8) based on colony and conidia shape attributes (Fig. 1). Thirty seven percent of all isolates belonged to morphotype I, which had white gray-colored colonies with a dark and gray conidial mass in the center. The results revealed that the isolates of Colletotrichum spp produced hyaline cylindrical conidia. Isolates belonging to morphotype II were 38%) and had cream-to- white colored colonies with a salmon - gray colored conidial mass in the center and fusiform tapered to a point in both ends (Fig. 1). Isolates belonging to morphotype III (25%) had mint cream-to - light orange mycelia-colored colonies with reverse plate having black to light gray-colored conidial mass in the center and cylindrical conidia with obtuse to slightly rounded ends (Fig. 1).

Significant ($p< 0.05$) variations were observed with respect to conidial dimensions among the isolates.

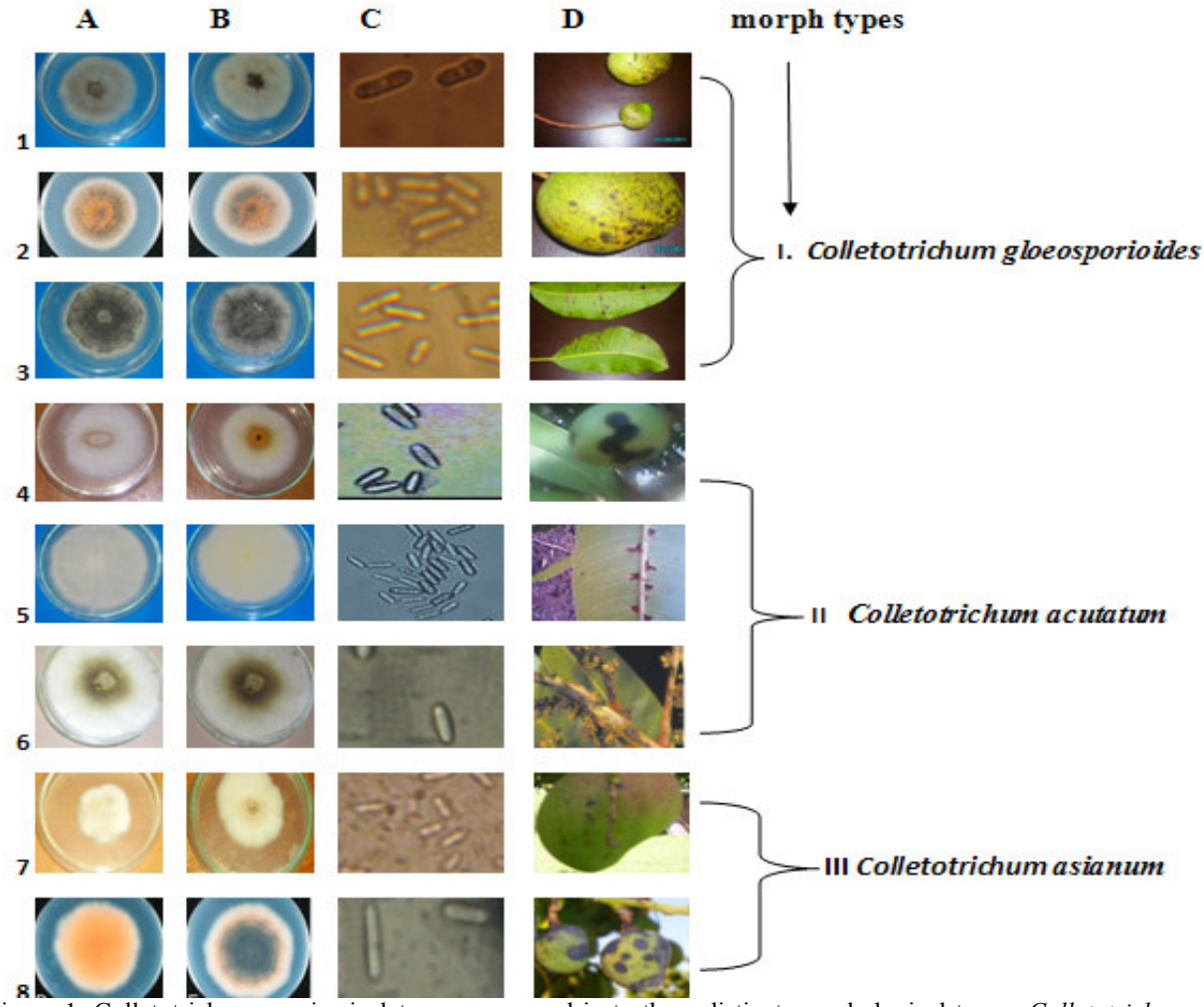

Figure 1. Colletotrichum species isolats were grouped in to three distinct morphological types: *Colletotrichum gloeosporioide* (morphotypes-I. isolate 1 to 3), *Colletotrichum acutatum* (morphotype -II. isolate 4 to 6) and *Colletotrichum asianum* (morpho types-III, isolats 7 and 8) based on colony and conidia characteristics: Plates in column A) aerial view; B) reverse view; C) typical conidia shape in microscopic view and D) infected mango plant organ from which Colletotrichum spices were isolated.

The length of conidia ranged from 10.5-17.8 µm. The highest length of conidia was observed in CAD-FR and CBK-L isolates (17.8 and 17.84 µm) followed by CG-L isolate (17.17 µm) and the shortest conidia was recorded in CMn-w isolates (10.5 µm).

Table.1 Colletotrichum spp isolates from different location of mango plant and their conidia characteristics

Isolate	conidia length			conidia width			conidia shape
	max	min	mean	max	min	mean	
CGm-FR	13.4	11.6	12.3	4.83	3.91	4.42	cylindrical
CAD-FR	17.8	13.83	15.42	5.52	4.6	5.01	cylindrical
CBK-FR	13.6	10.7	12.37	5.75	2.99	4.47	cylindrical
CMn-w	10.5	7.81	9.91	6.9	2.76	4.04	fusiform
CBm-T	14.3	11.15	12.95	5.52	3.22	4.39	fusiform
CG-L	17.17	11.82	14.64	5.98	4.6	5.05	fusiform
CBK-L	17.84	12.04	14.72	6.9	4.6	5.28	Cylindrical/obtuse end
CSC	14.94	10.04	12.6	5.98	4.14	4.83	Cylindrical/obtuse end

Width of the conidia ranged from 3.22 to 6.9 µm. Isolate CBK-L and CMn-w recorded the highest width of conidium (6.9 µm) and was followed by CSC, CG-L and CBK-FR with corresponding width of 5.98, 5.98 and5.75 µm, respectively. Lowest width was observed in CBm-T isolate (3.22 µm) (Table 1).The colony diameter and colony color were considered as growth characters. The results showed that all the six media tested supported the mycelial growth of the isolates of Colletotrichom spp

The highest mean colony diameter of 51.9 mm was on Potato dextrose agar followed by mango fruit

extract agar, Sabouraud dextrose agar and Malt extract agar with 41.1mm, 40mm, and 39.8mm, respectively. The isolates CG-L, CBK-FR, CMn-w and CAD-FR, colony diameter on potato dextrose agar were 88mm, 72mm, 71mm and 60mm, respectively. Isolate CAD-FR had the highest colony diameter on mango fruit extract agar (69mm) followed by potato dextrose agar (60mm) and malt extract agar (42mm) (Table 4).

Table.2 Cultural characteristics (colony color) of Colletotricum spp. isolates on different solid media

Isolate	TWA	SDA	PDA	MFEA	MLEA	MEA
CGm-FR	light gray	gray	white gray	light gray	light gray	gray
CAD-FR	gray	dark gray	orange gray	dim gray	light gray	pink orange
CBK-FR	gray	pink	gray	slight dark gray	light gray	gray
CMn-W	light cream	white	white	cream	beige brown	cream ring white
CBm-F	cream	light gold	creamy	cream	gray	gray
CG-L	gray	gray	white	light gray	slight gray	dark orange
CBK-L	gray	pink	Mint cream	light gray	dark gray	gray
CSC	gray	pink	Light orange	dim gray	dark gray	dark orange

Where, TWA= Tap water agar; SDA= Sabouraud dextrose agar; PDA= Potato dextrose agar; MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and MEA= Malt extract agar

All isolates had good sporulation on general media PDA followed by SDA and MEA. Isolate CSC had medium sporulation capacity on the SDA and MEA. CBM-T isolate had good sporulation on all media except on TWA whereas CMn-w isolate didn't sporulate on the TWA and MFEA (Table 3).

Table.10 Sporulation capacity of Colletotrichum spp identified from mango plants on different solid media

Isolate	TWA	SDA	PDA	MFEA	MLEA	MEA
CGm-FR	**	***	***	*	**	***
CAD-FR	***	***	***	**	*	***
CBK-FR	***	***	***	*	***	***
CMn-W	_	***	***	_	***	***
CBm-F	_	***	***	***	***	***
CG-L	***	***	***	***	**	***
CBK-L	***	***	***	**	**	*
CSC	_	**	***	**	*	**

Where, TWA= Tap water agar; SDA= Sabouraud dextrose agar; PDA= Potato dextrose agar; MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and MEA= Malt extract agar

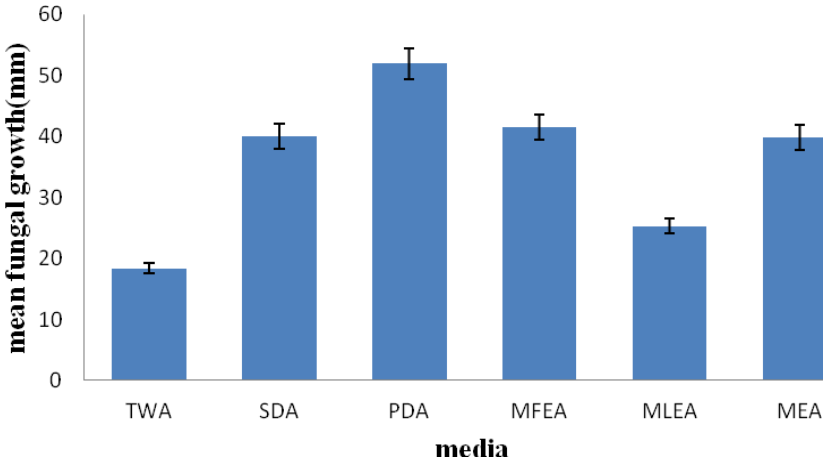

Figure 2. Mean mycelia growth of Colletotrichum species on different media. Where, TWA= Tap water agar; SDA= Sabouraud dextrose agar; PDA= Potato dextrose agar; MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and MEA= Malt extract agar

There was a statistically significant difference in mycelia growth among the eight isolates ($p < 0.001$) on different media. A lowest mean mycelia growth of 18.4 mm was recorded on TWA followed by MFEA 25.3mm (Fig 2).

Table 4. Effect of different solid media on the mycelia growth diameter (mm) of Colletotrichum spp isolates in Southwest Ethiopia.

Isolate	TWA	SDA	PDA	MFEA	MLEA	MEA
CGm-FR	30 [b]	44 [c]	25 [e]	41 [d]	35 [b]	27 [f]
CAD-FR	16[d]	30[f]	60[c]	69[a]	16[f]	42[c]
CBK-FR	14[e]	34[e]	72[b]	45[c]	21.5[e]	34[e]
CMn-w	4.1[g]	64[a]	71[b]	3[f]	13.5[g]	61[a]
CBm-T	36[a]	39[d]	25[e]	36[e]	26.3[d]	20[f]
CG-L	19[c]	51[b]	88[a]	39[d]	39.6[a]	55[b]
CBK-L	19[c]	33[e]	17[f]	50[b]	29.6[c]	33.3[e]
CSC	7[f]	23[g]	54[d]	48[b]	20.6[e]	39[d]
LSD	1.4	2.9	1.9	1.9	1.6	1.8
CV%	4.5	2.6	2.1	2.6	3.7	2.6

Column means followed by the same letter are not significantly different. Where, TWA= Tap water agar; agar; PDA= Potato dextrose agar; MEA= Malt extract MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and agar SDA= Sabouraud dextrose

Table 5. Growth rate (mm/day) of Colletotrichum spp on different solid media

Isolate	TWA	SDA	PDA	MFEA	MLEA	MEA
CGm-FR	4.3 [b]	6.3 [c]	3.6 [e]	5.8 [d]	5 [b]	3.8 [f]
CAD-FR	2.2[d]	4.3 [f]	8.7 [c]	9.8 [a]	2.2 [f]	6 [c]
CBK-FR	2 [e]	4.9 [e]	10.3 [b]	6.4 [c]	3 [e]	4.9 [e]
CMn-w	0.6 [g]	9.1 [a]	10.1 [b]	0.4 [f]	1.9 [g]	8.8 [a]
CBm-T	5.1 [a]	5.6 [d]	3.6 [e]	5.1 [e]	3.7 [d]	3.6 [f]
CG-L	2.8 [c]	7.3 [b]	12.6 [a]	5.6 [d]	5.6 [a]	7.8 [b]
CBK-L	2.8 [c]	4.8 [e]	2.5 [f]	7.1 [b]	4.2 [c]	4.8 [e]
CSC	1 [f]	3.2 [g]	7.5 [d]	6.9 [b]	2.9 [e]	5.5 [d]
LSD	0.2	0.27	0.28	0.28	0.23	0.25
CV%	4.4	2.7	2.1	2.7	3.7	2.6

Means followed by the same letter are not significantly different.
Where, TWA= Tap water agar; SDA=Sabouraud dextrose agar; PDA= Potato dextrose agar; MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and MEA= Malt extract agar Sabouraud dextrose agar; PDA= Potato dextrose agar; MLEA= Mango leaf extract agar; MFEA= Mango fruit extract agar and MEA= Malt extract agar

There was a statistically significant difference in growth rates among the eight isolates. Colletotrichum spp isolate CG-L grew significantly faster (12.6 mm/day), followed by CBK-FR and CMn-T, 10.3mm/day and 10.1mm/day respectively (Table 5). In general media (PDA) and the lowest growth rate (0.6mm/day) was recorded on CMn-w isolate on TWA media (Table.5).

Pathogenicity test

The results of the test revealed that all evaluated isolates caused typical anthracnose symptoms on the leaves, mango cultivars tested on Kent varieties. The sides of the leaves that served as controls did not show any symptoms. Some isolates were able to provoke extensive lesions on the leaves at inoculated points, whereas some isolates infected only small area of the inoculated points. Nearly all of the isolates provoked extensive, black, round lesions that were visible on both sides of the leaves. However, some isolates caused irregular, necrotic lesions surrounded by black spots, indicating the growth region of the fungus in the leaves tissue.

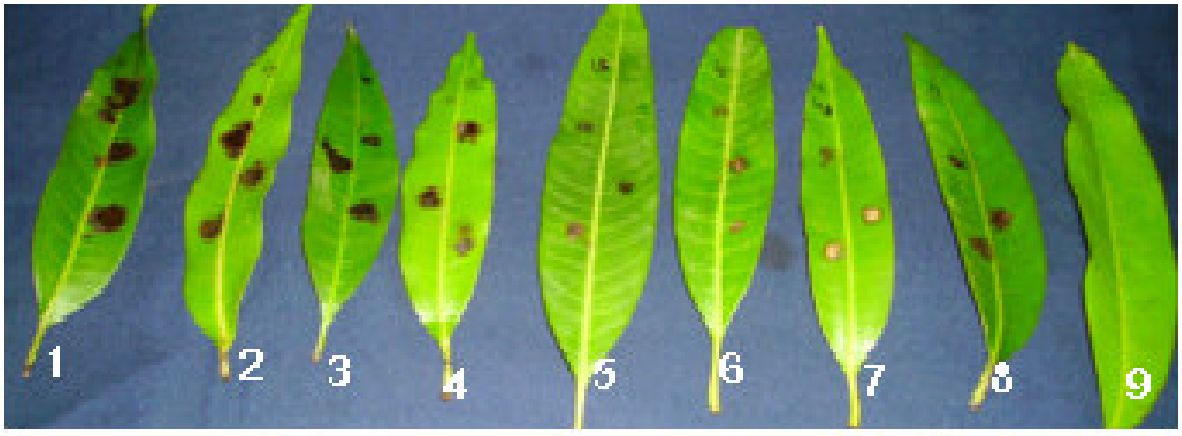

Figure 3. Pathogenicity tests with Colletotrichum isolates on mango leaves variety Kent. After 10 days incubation lesions caused by isolate 1) CGm-F, 2) CAd-F, 3) CBk-F, 4) CMn-w, 5) CBm-T 6) CGL, 7) CSC, and 8) CBL-L. 9) Controls. Isolate 1,2,and 3 characterized by large, rounded black spots, whereas the necrotic lesions caused by isolate 4,5,and 6, 7 and 8 were surrounded by necrotic and black spots, indicating the region of growth of the fungus in the leaf tissue. Acervuli were observed on the necrotic tissues.

Figure 4. Pathogenicity tests Colletotrichum isolate collected from mango leaf, panicles and immature attached fruit of southwest Ethiopia, inoculated on mango fruit variety Kent. 1) Inoculated detached mango fruit symptom after five days incubation 2) Before inoculation.

Acervuli and conidia were observed in the necrotic areas of the leaves (Fig. 3). Colletotrichum isolate inoculated on physiological mature unripe detached mango fruit were infected and lesion began as dark black lesions with circular to irregular spot gray center were observed around the inoculation (Fig. 4) point three day after inoculation.re-isolation of Colletotrichum isolates from inoculated fruits and leaves were consistence and confirmed the pathogenic role of this organism. Please move this paragraph to above Fig.4.

DISCUSSION

Colletotrichum spp. are an important genus of plant pathogenic fungi that cause pre and post-harvest rots and anthracnose on a wide range of fruit, vegetable and ornamental hosts, especially in subtropical and tropical regions (Hyde *et al.* 2009a). Recent studies showed that Colletotrichum spp. were listed in the top 10 fungal pathogens of scientific and economic importance (Dean *et al.,* 2012; Damm *et al*., 2013). There are many accounts of Colletotrichum spp surviving asymptomatically on plant surfaces (Leandro *et al.,* 2002; Talhinhas *et al*., 2011). Epiphytic and entophytic life phases and quiescent infection stages may precede a damaging necrotic phase in which lesions develop (Cannon *et al*. 2012).

In this investigation differences in colony characters, growth rates and conidial shapes among the Colletotrichum isolates made them to be separated into three morph type. *Colletotrichum gloeosporioides* produced dark grey colonies and formed typically cylindrical conidia with rounded ends on PDA. The other colonies were white to orange in color, with slight shades of pink and light mouse grey with very thick cottony aerial mycelium. On the reverse side, the centre was dark orange to pink, and the conidia produced were fusiform tapered to a point in both ends. colony appearance had mint cream-to - light orange mycelia-colored colonies with reverse plate having black to light gray-colored conidial mass in the center and cylindrical conidia with obtuse to slightly rounded ends this identified as *Colletotrichum asianum.*

Our observations confirmed by Damm *et al.* (2012) reliable characteristics to distinguish between *C.*

acutatum and *C. gloeosporioides* typical fusiform conidia and many cylindrical ones. Similarly, Jayasinghe & Fernando (2009) also reported for the first time the occurrence of *C. acutatum* on mango in Sri Lanka.

The present investigation revealed that the colony characters and growth of Colletotrichum isolates varied on different media. This might be due to the variation in the nutritional requirement of the fungus. Our result was in agreement with *C. acutatum* colony appearance described by Strandberg and Chellemi (2002) there was a wide variation in the colony characters colour, topography, pigmentation, sporulation and mycelial growth of different isolates even in the same media (Rani and Murthy, 2004). Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life process. Among Medias fungal pathogen from mango plant result showed that better growth and sporulates on Potato dexterous agar (PDA), Sabouraud dextrose agar (SDA), and Malt extract agar (MEA) respectively. Similar results were obtained by (Sudhakar, 2000; Rain and Mutthy, 2004; Tasiwal and Benagi, 2009) and Amarjit singh *et al*. (2006) who observed the maximum growth of *C. gloeosporioides* of Guava on PDA medium. Likewise, Jeyalakshmi and Seetharaman (1999) and Patil and Moniz (1973) reported that PDA was the best media for the growth and sporulation of *C. capsici.*

In the present study, the symptoms produced by the pathogen on artificial inoculation on the fruits were similar to the symptoms observed under natural infection. The symptom appeared as black, sunken lesions were distributed all over the outer part of the fruit. The fungus starts developing acervuli, with concentric rings and sporulating with mass of pinkish conidia. Severely affected fruits become blackened and rot or shrivel and mummified. The symptoms observed under artificial conditions agreed with same type of natural symptom. Similar symptom of anthracnose was also noticed on banana fruits with sunken lesions and covered with salmon colored acervuli (Stover and Simmonds, 1987).

In this study, Colletotrichum spp isolates from infected mango tissue were identified as *C. gloeosporioides,* C. *acutatum*, and *C. asianum* which demonstrate that in homestead orchard in Southwest Ethiopia. Similar findings confirmed by other researchers (Cannon e*t al*. 2000; Peres *et al*., 2002; Afanador-Kafuri *et al*., 2003; Photita *et al*., 2005; Cai *et al*. 2009; Silva-Rojas and Avila-Quezada, 2011) who reported that *C. gloeosporioides* is a common pathogen on a variety of tropical crops such as mango, avocado and papaya.

The results of the current study showed that there was no geographical specificity concerning colonies color of Colletotrichum spp isolated from mango, and similar previous results were reported by different authors (Rojas *et al.* 2010; Silva *et al*., 2012a, b). Jayasinghe and Fernando (2009) also reported the occurrence of *C. acutatum* on mango. A result of this study confirms the result reported by Prihastuti *et al.* (2009) in *Coffea arabica* from Thailand. *C. asianum* associated with the immature attached mango fruits in south west Ethiopia (Amsalu, *et al,* unpublished data). Strains of *Colletotrichum asianum* infected Chili, mango and rose apple host (Phoulivong *et al,* 2012). However, it is already known from *Mangifera indica* in Australia, Colombia, Japan, Panama and the Philippines (Weir *et al*. 2012; Lima *et al*., 2013).

The results of this study showed different species of Colletotrichum isolates occur on the same host, and this investigation was in agreement with the report of Sanders and Korsten, (2003); Prihastuti *et al.* (2009), Damm *et al.* (2012a) report members of the *C. acutatum* and *C. boninense* species complexes, *C. simmondsii, C. fioriniae*, and *C. karstii*, from mango from Australia (Prihastuti *et al.* 2009).

Conclusion

Fro results of the present study it can be concluded that, the information generated in this work is relevant and can assist in the implementation of disease control and prevention measures more effectively. Further study on molecular characterization and epidemiology of anthracnose of mango is vital for disease management strategies.

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